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14. ABSTRACT Post traumatic stress disorder (PTSD) is a complex clinical disorder resulting from exposure to intense, life-threatening events resulting in persistent re-experiencing of the trauma, avoidance of stimuli associated with the trauma, dissociation, and heightened arousal which severely impact social and occupational functioning. Recent work has underscored morphological and functional brain alterations in PTSD patients using brain imaging with MRI, SPECT, and PET imaging. Despite this encouraging preliminary work, there exists only a limited understanding of the pathophysiological changes which may subserve symptoms of PTSD. Preclinical studies now suggest that inflammatory changes may be implicated in neuronal loss in models of PTSD. Microglia, represent a key inflammatory cell mediator within the CNS. Upon activation, these cells densely express an18kDa translocator protein (TSPO) receptors on their cell surface. We have performed a study with the TSPO imaging agent 18-F PBR111 with the goal of this proposal is to explore a novel biomarker. Eight PTSD and six healthy volunteers participated in a single PET study following injection of 18-F PBR111. Preliminary findings suggest a reduction in regional brain distribution volume in PTSD compared to controls which correlates with clinical ratings of early psychic trauma.					
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Introduction

Activated microglia have been proposed to play a major role in the pathogenesis of several central nervous system conditions including post-traumatic stress disorder (PTSD) and traumatic brain injury (TBI). Microglia represent over 10% of the cells in the brain and become “activated” in response to various stimuli. This activation is thought to control areas of inflammation in the brain, although in certain neurological diseases, this activation may also contribute to ongoing neuronal damage. When microglia become activated they express peripheral benzodiazepine receptors (PBR), now referred to as the 18kDa translocator protein (TSPO), on their mitochondrial membranes. TSPO are functionally and structurally distinct from central benzodiazepine receptors associated with gamma-aminobutyric acid (GABA)-regulated chloride channels. TSPO are found in abundance in peripheral organs and hematologic cells, but are normally present at only very low levels in the central nervous system,

A number of ligands with high *in vitro* affinity for TSPO have been investigated for PET or SPECT imaging. To date, 11-C PK-11195 has been considered the best agent, in spite of its limitations. 123-I PK-11195 was also evaluated in humans but its low brain penetration makes it unfavorable for further evaluation. PET imaging using 11-C PK11195, a PBR agent, has demonstrated increased uptake in patients with several neurodegenerative conditions. Although 11-C PK11195 has provided critical proof of concept that *in vivo* imaging may be used to monitor neuroinflammation, there are several limitations to this ligand. Quantitation of 11-C PK11195 is difficult and use of 11-C labeled ligands are not practical in large clinical studies because of the short half-life (20 min). More recently, 18-F and 11-C labeled DAA1106 analogs were shown to be promising for imaging TSPO. These newer generation TSPO radiopharmaceuticals bind somewhat differently from PK11195, in that there are common genotypes in the population resulting in different binding profiles. Specifically, three different brain binding affinity patterns; high affinity binding, mixed affinity binding, and low affinity binding, were found for PBR28, PBR06, PBR111, DAA1106, and DPA713 (Owen et al., 2011). Different binding affinity is related to a single amino acid substitution, an Ala147Thr polymorphism. Approximately 10% of the population are low affinity binders, 50% are high affinity binders, and the remainder mixed affinity binders. Hence, it is critical to know the subject's binding status to accurately interpret the imaging data.

In this proposal we originally developed a TSPO-binding radiotracer labeled with 123-I (T_{1/2} 13.1 h) to enhance the clinical utility of imaging of neuroinflammatory targets. Unfortunately, 123-I CLINDE, the radiotracer initially proposed had limited signal:noise properties in other human trials. We have subsequently identified a better agent for interrogating TSPO in post-traumatic stress disorder (PTSD) subjects, 18-F PBR111, a PET compound with more favorable human preliminary data than 123-I CLINDE. PBR111 binds selectively to TSPO and increased PBR111 binding is a marker of CNS microglial activation in animal models of neuroinflammation. The increase in PBR111 binding is an indicator of the transition of microglia from a resting to an activated state, reflecting inflammation and its clinical sequelae which may be relevant to PTSD symptomatology.

Body

This is a Phase 1, single-center, open-label, non-randomized, clinical study in PTSD and healthy volunteers (HVs) to evaluate the kinetics, clearance and cerebral distribution of [18F]PBR-111 as outlined in the study protocol. Investigational new drug approval has been granted by the FDA to study this agent (IND # 107,622). The underlying goal of this study is to assess [18F]PBR-111 PET imaging as a tool to detect microglial activation in the brain of PTSD research participants compared with similarly aged healthy subjects. All study procedures were conducted at the Institute for Neurodegenerative Disorders (IND) in New Haven, CT.

A total of 8 subjects with a clinical diagnosis of PTSD and 6 healthy controls were recruited to participate in this study. The diagnosis of PTSD was confirmed based on the DSM-IV criteria after clinical and neuro-psychiatric examination. Similarly, healthy controls were evaluated for any evidence of significant neurological or psychiatric disturbance. Table 1 summarizes the demographic characteristics of the PTSD subjects and controls.

Table 1. Subject Demographics and Scores on Trauma Ratings

Healthy Controls	Age at Scan	Gender	Race	Early Trauma Inventory (ETI) - < age 18	Early Trauma Inventory (ETI) - TOTAL
PBR111-002-02	26.4	F	AA	4	7
PBR111-002-03	23.8	M	AA	20	24
PBR111-002-05	23.8	F	W	6	11
PBR111-002-08	29.9	M	AA	14	20
PBR111-002-10	30.4	M	AA	16	24
PBR111-002-12	50	M	W	5	17

PTSD Subjects	Age at Scan	Gender	Race	Early Trauma Inventory (ETI) - < age 18	Early Trauma Inventory (ETI) - TOTAL	Mississippi Scale for Combat PTSD (MISS)	Combat Exposure Scale (CES)
PBR111-002-06	67	M	W	16	26	104	34
PBR111-002-07	46	M	His	19	30	88	88
PBR111-002-15	48	M	W	16	29	149	34
PBR111-002-16	28	M	W	11	25		
PBR111-002-18	36	M	W	7	20	122	30

PBR111-002-20	28	M	W	3	7	100	26
PBR111-002-21	65	M	W	28	44	120	25
PBR111-002-23	37	M	W	14	24	142	38

Each research volunteer participated in a single PET study following injection of [18F]-PBR111 as outlined in the study protocol. Briefly, each PET study was acquired as a series of dynamic PET frames to characterize the regional brain uptake and washout of radiotracer. Following bolus injection of [18]-F PBR111, serial dynamic PET brain acquisitions were obtained for three hours. These dynamic PET datasets underwent pharmacokinetic modeling using classic two-tissue compartment models with a full metabolite-corrected arterial input function obtained during arterial blood sampling. Images were reconstructed with a standard iterative algorithm with scatter, randoms, and attenuation corrections applied. Each serial image volume was spatially normalized and co-registered with a T1 weighted MRI and a standardized volume of interest brain template based on the AAL template was checked for accuracy on the MRI then transferred to the PET image for extraction of standard uptake values (SUV). These regional brain SUVs were then modeled with classic two tissue (2T), one tissue (1T), and Logan plot kinetic analyses (Fig 1) to determine the regional brain distribution volume, V_t . In addition, an exploratory, pixel-wise analysis (2T) was performed on the image volumes using modeled constraints determined in the VOI-level analysis. Regional brain V_t values were compared between groups after correction for TSPO binding status. In addition, within the PTSD cohort correlations were performed between regional brain V_t and trauma ratings.

Example of modelling fits investigated

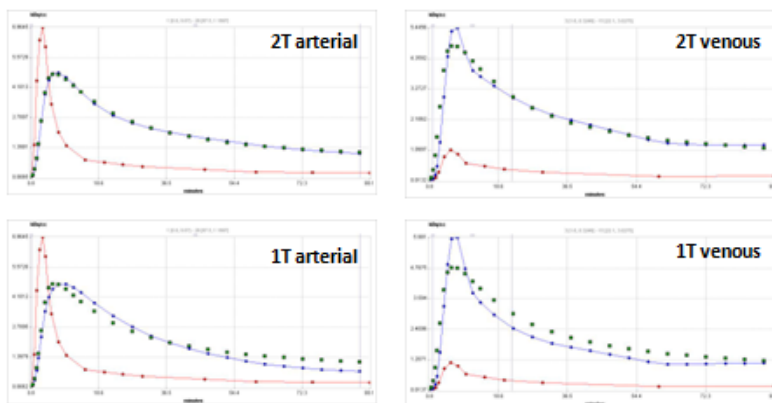


Figure 1 Example of different kinetic models for determining brain distribution volumes (V_t) using arterial vs venous blood and two-tissue (2T) vs one tissue (1T) brain compartmental models. The 2T model with arterial blood provided the best fit with the PET data and was used in the present investigation.

Key Research Accomplishments

Following a slow start to the study which was driven by need to change the radiopharmaceutical to be used for assessing neuroinflammation, we have completed the trial as outlined in the study protocol. This report summarizes the completed recruitment and initial analyses of these data. Summarizing this, objective progress in this PTSD trial includes:

1. Completed enrollment, PET imaging, and other data collection in all PTSD and healthy volunteers proposed in the grant.
2. Performed full two tissue compartment pharmacokinetic modeling of the PET data as well as explored other invasive and non-invasive kinetic models using standardized region of interest analyses.
3. Developed a method to obtain pixel-wise distribution volume PET image maps to explore clustered V_t voxels without a *priori* regional bias.
4. Initiated full statistical interrogation of the of the PET datasets comparing TSPO binding between cohorts as well as correlations within the PTSD cohort between regional PET V_t and clinical trauma ratings.

The issues and work delineated above are described in the subsequent portion of this report. Given the amount of data collected, only a highlight summary is provided in this report.

Reportable Outcomes

These studies are performed under IND # 107,622 at the Institute for Neurodegenerative Disorders, New Haven, CT. All subjects tolerated the PET procedures without significant subjective or objective changes following radiotracer injection. All 14 PET scans (8 PTSD participants and 6 healthy volunteers) were of good quality and useable for the analysis, including acquisition of full arterial blood data for kinetic modeling of each PET scan using metabolite-corrected arterial input functions for calculation of regional brain distribution volume, V_t , in standard two-tissue compartment models.

Metabolism of PBR111 There were no statistically significant differences in the metabolism of the parent 18-F PBR111 or the amount of plasma protein bound parent compound (Fig. 2) in PTSD participants and healthy volunteers. Similar to other human investigations with 18-F PBR111, this study demonstrates that parent compound is quickly metabolized with residual parent reflecting 15-30% of activity at 90 minutes. In addition, PBR111 is primarily metabolized to two extractable metabolites- one of which is predominant (Metabolite A= 50-90%, Metabolite B = 0-30%), without evidence of lipophilic metabolites which can confound the analysis.

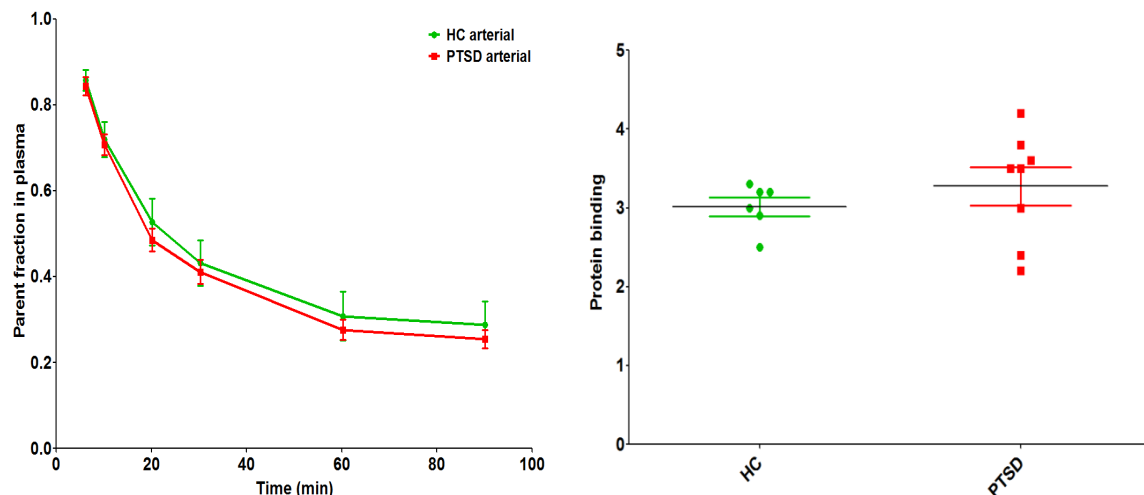


Figure 2 Left graph: Mean PTSD and healthy volunteers plasma fraction of parent 18-F PBR111 over time demonstrates no statistical differences in metabolism between the cohorts. Right graph: There is similar plasma protein binding of 18-F PBR111 in healthy and PTSD participants. Individual subject data are indicated on the graph.

TSPO Binder status Both mixed and high affinity TSPO binders were evident in the PTSD (4 high affinity binders, 4 mixed affinity binders) and healthy (3 high affinity binders, 3 mixed affinity binders) cohorts. No low affinity binders were noted. PET data (V_t) from mixed and high affinity binders were combined following multiplication by the correction factor of 1.5 to the mixed binders consistent with other studies and following an empirical check of the control data for each brain region to assess this correction factor. Analyses were done both with the combined binder groups and individually for high affinity and mixed affinity groups.

Distribution Volume Measures Significant differences were noted in brain 18F PBR111 distribution volume (V_t) measures in PTSD and healthy volunteers across multiple brain regions (Fig 3). In each instance PTSD participants demonstrated *lower* V_t measures than healthy volunteers with statistically significant differences noted in cingulum, hippocampus, amygdala, globus pallidus, putamen, thalamus, temporal cortex, cerebellum, and brainstem. Figure 4 shows 18-F PBR111 distribution volume ratios (V_t) in amygdala and hippocampus in individual PTSD and healthy volunteers for both high and mixed affinity TSPO binders using the correction factor noted above.

Correlations between 18-F PBR111 binding and clinical trauma ratings were performed showing several highly correlated findings. As example, Figure 5 demonstrates the good correlation between amygdala V_t and early trauma exposure measured by the ETI for high and mixed affinity binder PTSD. There was relatively poor correlation ($R^2 = .02$) between amygdala V_t and the Mississippi Scale for Combat PTSD.

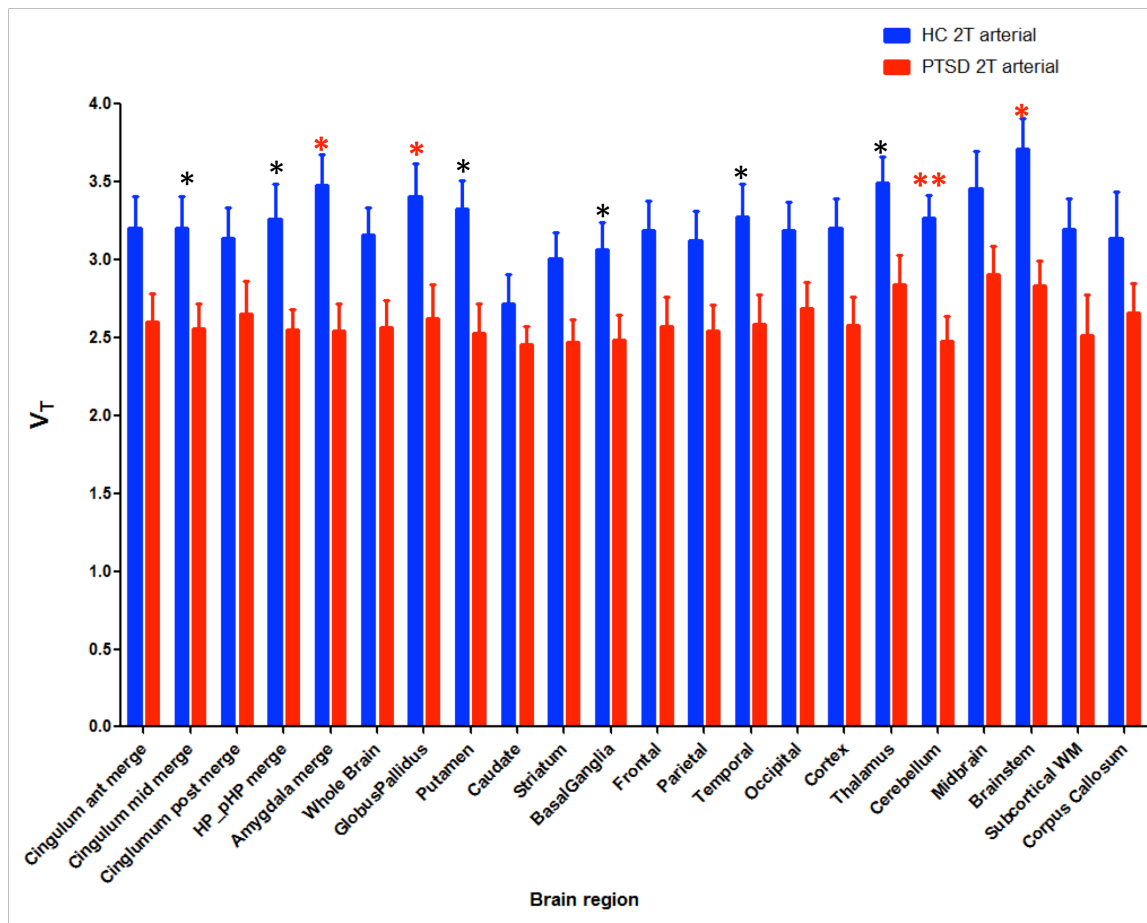


Figure 3 18-F PBR111 distribution volume ratios (V_t) in PTSD and healthy volunteer high affinity TSPO binders. Healthy controls have consistently higher V_t across brain regions.

Our original hypothesis anticipated increases in V_t brain regions, especially temporal and limbic regions indicating an acute neuroinflammatory process. As this was not the case, we evaluated potential sources of error which could lead to the current findings. As already mentioned above, there were no differences in the metabolism of the radiotracer between the controls the PTSD group. In addition, blood flow to brain was not different based on comparison of the kinetic rate constant describing the rate of brain radiotracer influx, K_1 , ascertained during kinetic modelling of the PET data. Pharmacokinetic modeling takes into consideration factors like metabolism and bloodflow in determining the specific binding of the tracer at the TSPO site. Another potential consideration is the possible differences in cortical atrophy between the PTSD subjects in healthy volunteers. Atrophy produces partial volume errors and can lower distribution volume measurements. Atrophy correction is pending in this dataset, but there is no strong evidence of structural brain differences in the cohorts to serve as explanation for the findings.

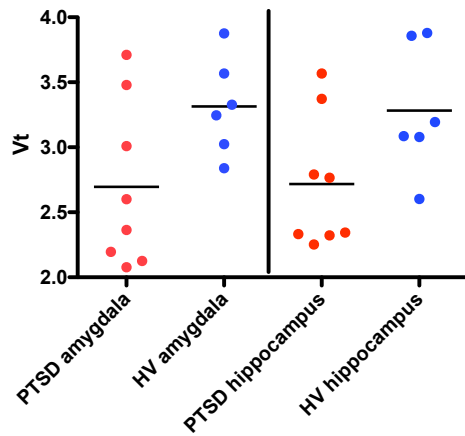


Figure 4 18-F PBR111 individual distribution volume ratios (Vt) in amygdala and hippocampus in PTSD and healthy volunteer for both high and mixed affinity TSPO binders.

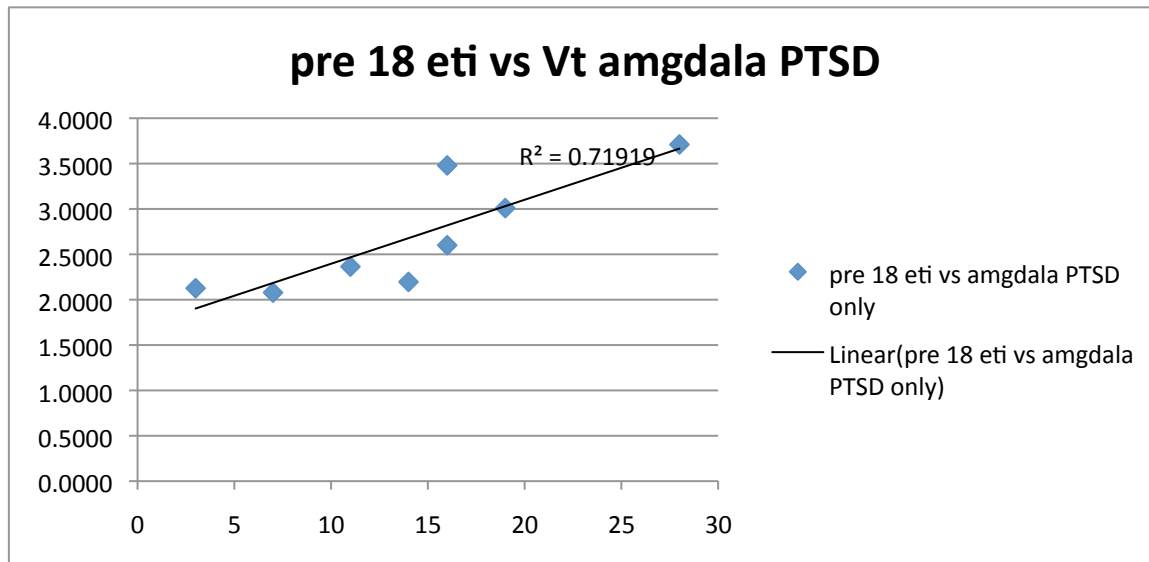


Fig 5 Correlation between early trauma measured by the Early Trauma Inventory and amygdala Vt in eight PTSD participants.

A general role for neuroinflammation in normal neuronal function and in brain disease has been difficult to glean from the wide variety of published clinical and preclinical studies. A unifying model is emerging in which there is a “right amount” of glial activation for various states, but that too much or too little is invariably detrimental. Activated glial cells, especially microglia, are involved in modulating neuronal signaling and release an array of both trophic and cytotoxic factors. For example, driving excessive activation, as occurs in HIV-related dementia, clearly impairs neuronal function and eventually leads to neuron death. On the other hand, preclinical models of disorders such as amyotrophic lateral sclerosis or Alzheimer’s dementia demonstrate that preventing microglial activation accelerates neuron loss and functional disability. A low level of activation is required in the normal CNS. This must be acutely elevated in response to brain insults to protect the neurons or eliminate pathogens or toxins. If the inflammatory response, however, is too robust or prolonged, permanent neuronal damage occurs.

In PTSD, it may be that both pathological states occur over time. Several studies have shown that severe stress triggers medial temporal lobe microglia-mediated neuroinflammation. Different individuals are prone to more robust or prolonged response to the similar stimuli as others. After an acute and subacute phase, the neuroinflammation subsides. In some instances, microglial markers then fall below baseline. This loss of microglial elements may account for some of the hippocampal volume loss that has been reported among PTSD subjects. Without the appropriate level of glial support, the trophic support and excitatory neurotransmitter buffering capacity would be lost. This would be expected to lead to impaired temporal lobe function and response-hyperreactivity.

Conclusion

In summary, this preliminary analysis in a feasibility study of PTSD and healthy volunteer suggests [18]F PBR111 as a marker of TSPO is reduced in PTSD across multiple brain regions, especially temporal lobe and limbic regions. These reductions correlate with clinical ratings of early psychological trauma. These data *are not* consistent with an acute neuroinflammatory process in long-standing PTSD, but suggests a more nuanced change in microglial response and neuroinflammatory changes. Contrary to our initial hypothesis of an expected increased signal in temporal and limbic brain regions, our data suggests that subjects with PTSD may in fact have persistently reduced microglial support. This would suggest that while initial therapies aimed at reducing neuroinflammation could be useful, longer term augmentation of the supportive functions of microglia could be beneficial.

References

1. Marín-Teva, J.L., et al., *Microglia and neuronal cell death*. Neuron Glia Biology, 2012. **FirstView**: p. 1-16.
2. McGeer, P.L. and E.G. McGeer, *Glial reactions in Parkinson's disease*. Movement Disorders, 2008. **23**(4): p. 474-483.
3. Chen, M.K. and T.R. Guilarte, *Translocator protein 18 kDa (TSPO): molecular sensor of brain injury and repair*. Pharmacol Ther, 2008. **118**(1): p. 1-17.

4. Schwartz, M., *Macrophages and microglia in central nervous system injury: are they helpful or harmful?* J Cereb Blood Flow Metab, 2003. **23**(4): p. 385-94.
5. Tilleux, S. and E. Hermans, *Neuroinflammation and regulation of glial glutamate uptake in neurological disorders.* J Neurosci Res, 2007. **85**(10): p. 2059-70.
6. Smith, J.A., et al., *Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases.* Brain Research Bulletin, 2012. **87**(1): p. 10-20.
7. Walter, L. and H. Neumann, *Role of microglia in neuronal degeneration and regeneration.* Semin Immunopathol, 2009. **31**(4): p. 513-25.
8. Brown, G. and J. Neher, *Inflammatory Neurodegeneration and Mechanisms of Microglial Killing of Neurons.* Molecular Neurobiology, 2010. **41**(2): p. 242-247.
9. Verkhratsky, A. and F. Kirchhoff, *Glutamate-mediated neuronal-glia transmission.* J Anat, 2007. **210**(6): p. 651-60.
10. Gras, G., et al., *The glutamate-glutamine cycle as an inducible, protective face of macrophage activation.* J Leukoc Biol, 2006. **80**(5): p. 1067-75.
11. Kaul, M., G.A. Garden, and S.A. Lipton, *Pathways to neuronal injury and apoptosis in HIV-associated dementia.* Nature, 2001. **410**(6831): p. 988-994.
12. Rock, R.B., et al., *Role of Microglia in Central Nervous System Infections.* Clin. Microbiol. Rev., 2004. **17**(4): p. 942-964.
13. Chiu, I.M., et al., *T lymphocytes potentiate endogenous neuroprotective inflammation in a mouse model of ALS.* Proceedings of the National Academy of Sciences, 2008. **105**(46): p. 17913-17918.
14. Kang, J. and S. Rivest, *MyD88-deficient bone marrow cells accelerate onset and reduce survival in a mouse model of amyotrophic lateral sclerosis.* J. Cell Biol., 2007. **179**(6): p. 1219-1230.
15. Lastres-Becker, I., et al., *α -Synuclein expression and Nrf2 deficiency cooperate to aggravate protein aggregation, neuronal death and inflammation in early-stage Parkinson's disease.* Human Molecular Genetics, 2012.
16. Beers, D.R., et al., *Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis.* Proceedings of the National Academy of Sciences, 2006. **103**(43): p. 16021-16026.
17. Fuller, S., M. Steele, and G. Munch, *Activated astroglia during chronic inflammation in Alzheimer's disease--do they neglect their neurosupportive roles?* Mutat Res, 2010. **690**(1-2): p. 40-9.
18. Henkel, J.S., et al., *Microglia in ALS: the good, the bad, and the resting.* J Neuroimmune Pharmacol, 2009. **4**(4): p. 389-98.
19. Srebro, Z. and K. Dziobek, *Neuroprotection: the role of neuroglia.* Folia Med Cracov, 2001. **42**(3): p. 113-21.
20. Appel, S.H., et al., *The microglial-motoneuron dialogue in ALS.* Acta Myol, 2011. **30**(1): p. 4-8.
21. Avital, A., et al., *Acute and repeated swim stress effects on peripheral benzodiazepine receptors in the rat hippocampus, adrenal, and kidney.* Neuropsychopharmacology, 2001. **25**(5): p. 669-78.
22. Drugan, R.C., et al., *Environmentally induced changes in peripheral benzodiazepine receptors are stressor and tissue specific.* Pharmacology Biochemistry and Behavior, 1995. **50**(4): p. 551.
23. Lehmann, J., et al., *Peripheral benzodiazepine receptors in cerebral cortex, but not in internal organs, are increased following inescapable stress and subsequent avoidance/escape shuttle-box testing.* Brain Res, 1999. **851**(1-2): p. 141-7.

24. Aardal-Eriksson, E., et al., *Salivary cortisol and serum prolactin in relation to stress rating scales in a group of rescue workers*. Biological Psychiatry, 1999. **46**(6): p. 850-855.
25. Maia, S., et al., *Longitudinal and parallel monitoring of neuroinflammation and neurodegeneration in a 6-hydroxydopamine rat model of Parkinson's disease*. Synapse, 2012: p. n/a-n/a.
26. Rupprecht, R., et al., *Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders*. Nat Rev Drug Discov, 2010. **9**(12): p. 971-88.
27. Da Pozzo, E., B. Costa, and C. Martini, *Translocator protein (TSPO) and neurosteroids: implications in psychiatric disorders*. Curr Mol Med, 2012. **12**(4): p. 426-42.

Appendix

None